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# Surface display of sialyltransferase on the outer membrane of *Escherichia coli* and ClearColi

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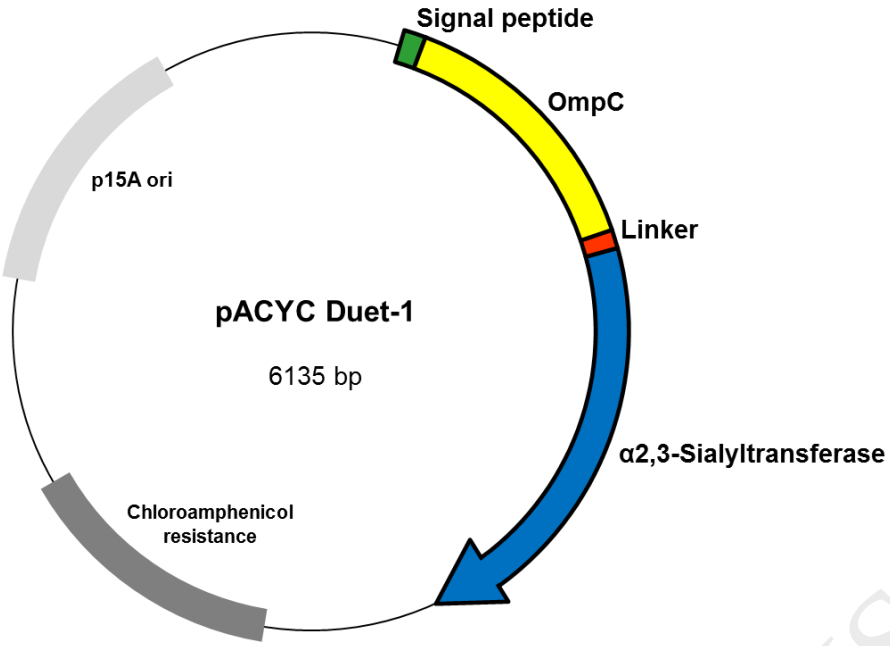
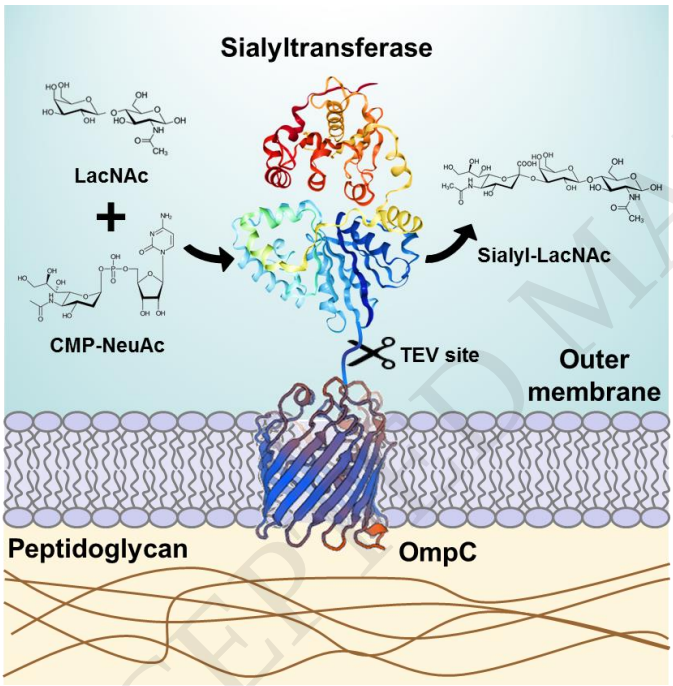
## Graphic abstract




## Highlights

- Sialyltransferase (PmST1) was surface displayed by using OmpC as an anchor protein.
- Expression levels of PmST1 on wildtype the outer membrane of *E. coli* (BL21) and

ClearColi were compared.

- Enzyme activity of both PmST1s was measurement of product concentration of CMP.
- The differences in kinetic parameters ($V_{max}$, $K_M$, $k_{cat}$)of PmST1 from both strains were estimated.

**Abstract**

α2,3-Sialyltransferase from *Pasteurella multocida* (PmST1) is an enzyme that transfers a sialyl group of donor substrates to an acceptor substrate called N-acetyl-D-lactosamine (LacNAc). In this study PmST1 was expressed on the outer membrane of wildtype *Escherichia coli* (BL21) with lipopolysaccharide (LPS) and ClearColi with no LPS, and then the enzyme activity and expression level of PmST1 were compared. As the first step, the expression levels of PmST1 on the outer membranes of wildtype *E. coli* (BL21) and ClearColi were compared according to the IPTG induction time, and the absolute amount of surface-displayed PmST1 was calculated using densitometry of SDS-PAGE. As the next step, the influence of LPS on the PmST1 activity was estimated by analyzing Michaelis-Menten plot. The enzyme activity of PmST1 was analyzed by measuring the concentration of CMP, which was a by-product after the transfer of the sialyl group of donor compounds to the acceptor compounds. From a Michaelis–Menten plot, the enzyme activity of the surface-displayed PmST1 and the maximum rate ($V_{max}$) of ClearColi were higher than those of wildtype *E. coli* (BL21). However, the $K_M$ value, which

represented the concentration of substrate to reach half the maximum rate ($V_{max}$), was similar for both enzymes. These results represented such a difference in enzyme activity was occurred from the interference of LPS on the mass transport of the donor and acceptor to PmST1 for the sialyl group transfer.

**Keywords:** sialyltransferase, surface display, ClearColi, OmpC, N-acetyl-D-lactosamine (LacNAc)

## 1. Introduction

Sialic acids comprising a family of over 40 neuraminic acid derivatives are involved in many biological and pathological phenomena by occupying the terminal position on macromolecules and cell membranes [1-3]. Sialylation through chemical synthesis is one of the most difficult glycosylation reactions because of the tertiary anomeric center of the sialic acid group [4]. Chemo-enzymatic synthesis has also been used for sialylation by using sialyltransferase (ST, EC 2. 4. 99. 4), which transfers sialic acid to the terminal of the glycan chains of proteins [5]. Usually, a nucleotide sugar called cytidine-5′-monophospho-N-acetylneuraminic acid (CMP-Neu5Ac) is used as a donor to supply sialic acid to acceptors, i.e., the glycan chains of proteins [2]. STs have been found in many bacterial strains, including *Neisseria gonorrhoeae*, *Photobacterium damsel*, and

*Pasteurella multocida*, and more than 18 types of human STs have been reported according to the expression organ (brain, liver, placenta, pyloric mucosa, etc.) and the type of glycosylation (α2,3, α2,6, or α2,8) [6-9]. Bacterial STs are well overexpressed in *Escherichia coli.* α2,3-sialyltransferase from *Pasteurella multocida* (PmST1) has been widely used for the production of various types of sialylation products because of its high expression level and multi-functional properties [10]. In particular, sialyl-Lewis X is a human cancer biomarker, which can be made through the sequential reactions of PmST1 and α1,3/4-fucosyltransferase [3, 11].

Surface display is a protein expression technology that expresses a target protein on the cell surface. In surface display, the target protein is genetically combined with the carrier proteins (anchoring motifs), which are finally expressed on the cell surface together with target proteins (Fig. 1(a)). Thus far, surface display has been widely used in the field of biotechnology including whole-cell biocatalysts, the production of hormones, and biosensors [12, 13].

Many proteins on the cell surface have been used as carrier proteins (anchoring motifs), including surface appendages [14, 15], lipoproteins (Ice Nucleation Protein, TraT, PAL, etc.) [16, 17], virulence factors (AIDA-1, EaeA, etc.) [18], and outer membrane proteins (OMPs) [19, 20]. Various OMPs have been used as carrier proteins, such as Omp1, OmpA, OmpC, OmpT, and OprF. OMPs have transmembrane β-barrels, which have short loops at the periplasmic side and long loops at the external side of the outer membrane of *E. coli*. Given that the external loops are generally less conserved and more susceptible to insertions and deletions of partial amino acid sequences, the target proteins have been inserted at the external C-terminal region of the β-barrel [13]. Therefore, surface display

based on OMPs has been used mainly for inserting small peptides to maintain the stability of the β-barrel. Among various OMPs, OmpC has the capacity to insert relatively large proteins with molecular weights of 18–52 kDa at the second external loop, using the C-terminal deletion-fusion method (Fig. 1(b)), and the expressed enzymes are known to have high stability [13, 20, 21].

The outer membrane of gram-negative bacteria such as *E. coli* is covered with lipopolysaccharide (LPS), and antibodies against the *E. coli* outer membrane are believed to target the LPS layer as antigenic sites [22-24]. LPS-free *E. coli* do not allow the formation of LPS in their outer membrane. The synthesis of LPS in these bacteria is modified through the incorporation of seven genetic deletions (ΔgutQ, ΔkdsD, ΔlpxL, ΔlpxM, ΔpagP, ΔlpxP, and ΔeptA), which remove all the carbohydrate decorations usually attached to the LPS (Fig. 1(c)) [25-29]. In this study, we aimed to assess the impact of LPS on the expression and activity of an enzyme as the first step to the development of cell surface-based biocatalysts. Thus, the enzyme activity as well as the expression level of PmST1 was compared when PmST1 is expressed on the outer membrane of a wildtype *E. coli* (BL21) with LPS and ClearColi with no LPS. As the first step, the expression levels of PmST1 on the outer membranes of the wildtype *E. coli* (BL21) and ClearColi were compared according to the IPTG induction time, and the absolute amount of surface-displayed PmST1 was analyzed using densitometry of SDS-PAGE. Then, the enzyme activity of PmST1 was estimated by measuring the concentration of CMP, which is a by-product after the transfer of the sialyl group to the acceptor.

## 2. Materials and methods

### 2.1 Materials

High salt Luria-Bertani (LB) medium was purchased from Duchefa (Haarlem, Netherlands). The pACYC Duet-1 expression vector was purchased from Novagen (Madison, WI, USA). Oligonucleotides were synthesized by Macrogen, Inc. (Seoul, Korea). Pierce™ BCA protein kit, high-fidelity Phusion polymerase, and all other PCR reagents were purchased from Thermo Fisher Scientific, Inc. (Waltham, MA, USA). N-Acetyl-D-lactosamine (LacNAc) and CMP-N-acetylneuraminic acid sodium salt (CMP-Neu5Ac) were purchased from Carbosynth (Compton, Berkshire, UK). Tetrabutylammonium hydrogen sulfate (TBAH) was purchased from Sigma-Aldrich (Seoul, Korea).

### 2.2 Preparation of surface display vector and expression

For the surface display of PmST1, competent cells of intact *E. coli* BL21 (DE3) were transformed with a surface display vector (pACYC Duet-1). As shown in Fig. 1(a), the surface display vector was constructed by adding OmpC, a signal peptide for OmpC, a linker region between β-barrel and PmST1, and a PmST1 sequence between Nde I and Xho I sites [20, 21]. Then, *E. coli* strain BL21 (DE3) and LPS-free ClearColi were grown in a high salt LB medium (20 mL) containing chloramphenicol at a concentration of 100 μg/mL at 37 °C overnight. For the main culture, *E. coli* cells were incubated in a high-salt LB medium (300 mL) containing chloramphenicol (100 μg/mL) and β-mercaptoethanol (10 mM) at 37 °C with shaking, until $OD_{600\ nm}$ reached 0.6. To express the OmpC-PmST1, 0.1 mM isopropyl-d-1-thiogalactopyranoside (IPTG) was added to the culture, which was grown at 20 °C for 16 h under shaking condition [18, 28].

For the preparation of the outer membrane (OM), the collected E. coli cell pellets were resuspended in 60 mM Tris/HCl buffer (pH 8.0) with lysozyme (200 μg/mL), 20 mM sucrose, and 0.2 mM EDTA, and then incubated for 10 min at room temperature (25 °C). Then, extraction buffer (2 % Triton X-100, 50 mM Tris-HCl, and 10 mM $MgCl_2$), with 1 mM phenylmethylsulfonyl fluoride (PMSF), and aprotinin (20 μg/mL) were added for 25 min at 4 °C. The cell lysate was centrifuged at 17000 g for 5 min at 4 °C [30]. After the supernatants were again centrifuged at 48,640 g for 10 min at 4 °C, the pellets were washed twice with 10 mM pH 7.4 HEPES buffer, and then resuspended in the assay buffer (10 mM pH 7.4 HEPES buffer, 10 mM MnCl2, 100 μL). In the previous work [31], the isolated OM fraction was tested to check whether other proteins from cytoplasm (CP), periplasm (PP), and inner membrane (IM) of *E.coli* were present here. From the activity assay of marker proteins of β-galactosidase of CP, β-Lactamase of PP and NADH oxidase of IM showed that the amount of mixed marker proteins were estimated to be less than 1 % and the isolation yield of the OM was estimated to be 80 % [31].

**2.3 Densitometry**

To quantitate the expressed OmpC-PmST1 protein on the OM of *E. coli*, densitometry was performed using protein bands of SDS-PAGE gel. As the first step, the density of a protein band of OmpC-PmST1 was compared with standard protein bands with known concentrations [32-34]. In this study, bovine serum albumin (BSA, 66 KDa) was used as a standard protein since it had a similar molecular weight to OmpC-PmST1 (80 KDa) [32]. The concentrations of BSA were set to 250, 200, 150, 100, 50, and 25 μg/mL to obtain the standard protein bands. The densities of the BSA and OmpC-PmST1 bands were

analyzed using a gel documentation system (Chemi-Doc XRS), and then the protein concentration was calculated using Quantity-One software. The total protein concentration of the OM of BL21 (DE3) and LPS-free ClearColi was calculated using a BCA assay kit purchased from Pierce/Thermo Fisher Scientific, Inc (Waltham, MA, USA).

**2.4 PmST1 assay**

The activity of surface displayed OmpC-PmST1 was analyzed by quantification of the PmST1 reaction product (CMP), using high performance liquid chromatography (HPLC). All sialylation reactions were performed by mixing LacNAc, CMP-Neu5Ac, and the isolated OM with surface-displayed OmpC-PmST1 in an assay buffer. The reaction mixture was incubated at 37 °C for 10 min. After then the reaction mixture was placed in liquid nitrogen for 3 min, and then in boiling water for 2 min. The reaction mixture was then filtered and analyzed using HPLC. For HPLC analysis, Kinetex C18 reversed phase column from Phenomenex (Torrance, CA, USA) was used with Buffer A (10 mM phosphate buffer [pH = 6.5], 8 mM TBAH) and methanol as the mobile phase [35]. The flow rate was set to 1 mL/min. The mobile phase composition was programmed to be at 100 % Buffer A for the first 5 min (0–5 min), and the methanol percentage was increased up to 30% for 10 min (5–15 min) by linear gradation, and then the composition was maintained (Buffer A:methanol = 7:3) for 10 min (15–25 min). Reactants and products were detected using a UV detector at a wavelength of 254 nm. The amount of reaction product (CMP) according to the enzyme reaction time was analyzed by setting the reaction time to be 0, 2.5, 5, 10, and 20 min. The OM from 2.56 x $10^9$ cells was used for the enzyme reaction of 1.05 mM LacNAc (400 μg/mL) and 0.157 mM CMP-Neu5Ac (100

μg/mL). For the analysis of reaction rate according to the amount of OM, the reaction time was set to 10 min. The amount of the OM corresponding to 0, 1.28, 2.56, 3.84, and 5.12 x $10^9$ *E. coli* cells was used for 1.05 mM LacNAc (400 μg/mL) and 0.157 mM CMP-Neu5Ac (100 μg/mL). For the Michaelis–Menten plot, the reaction time was fixed at 10 min and the OM for 3.76 x $10^9$ *E. coli* was used for the enzyme reaction. The amount of OmpC-PmST1 was obtained based on the ratio of OmpC-PmST1 among OM total proteins calculated by densitometry. As substrates for the enzyme reaction, 0.314 mM CMP-Neu5Ac (200 μg/mL) and LacNAc at concentrations of 0, 0.26, 0.52, 0.78, 1.04, 1.56, and 2.08 mM were used. The sample without an acceptor (no LacNAc) was used as a control to calculate the donor hydrolysis activity of PmST1. The sialylation rate and kinetic parameter were calculated by subtracting the calculated control hydrolysis rate value from each experimental data. The kinetic parameters were obtained by fitting the experimental data into the Hill formula equation using Origin 9.0 [36, 37].

## 3. Results and discussion

### 3.1 Surface display of PmST1 on the OM of wildtype *E. coli* and ClearColi

PmST1 is an enzyme that transfers the sialyl group of a donor substrate to an acceptor substrate. As shown in Fig. 1(a), PmST1 was surface displayed as a fusion protein of OmpC, which is known to be the OM protein of *E. coli*, using a signal peptide of 21 amino acids. The gene of PmST1 of 398 amino acids was inserted to the surface display vector (pACYC Duet-1), and it was connected to the OmpC through a linker sequence with 18 amino acids (GGGGGGGGGGAENLYFQG). As shown in Fig. 1(b), the binding sites for the donor (CMP-Neu5Ac) and the acceptor (LacNAc) were known to be located at

several regions of the primary sequence of PmST1. In this study, the enzyme activity and expression level of PmST1 were compared, when PmST1 was expressed on the OM of wildtype *E. coli* (BL21) with lipopolysaccharide (LPS) and ClearColi without LPS. The molecular weight of PmST1 was estimated to be 48 kDa, and that of the fusion protein with OmpC was calculated to be 80 kDa. As shown in Fig. 1(d), SDS-PAGE results from the isolated *E. coli* OM proteins showed that PmST1 was clearly observed to be surface displayed on the OM of wildtype *E. coli* (BL21) and ClearColi, based on a comparison with a negative control of wildtype *E. coli* without the surface display vector.

The expression level of PmST1 on the OM of wildtype *E. coli* (BL21) and ClearColi was compared according to the IPTG induction time. For the quantitative comparison of the expressed fusion protein (OmpC-PmST1), the number of *E. coli* was controlled at 1.0 x $10^9$ cells for both wildtype *E. coli* (BL21) and ClearColi. As shown in Fig. 2(a), the induction time was set to 1, 3, 6, and 16 h for both strains in lane 1 (6), 2 (7), 3 (8), and 4 (9), respectively. The expressed level of PmST1 was compared using only the isolated OM proteins. The level of expression was normalized using the protein band of OmpA as a reference. As shown in Fig. 2(b), the expression level of PmST1 on the OM of ClearColi was observed to be higher than that of the wildtype *E. coli* (BL21) until the induction time of less than 6 h. However, the expression levels of both strains were observed to approach the same level after the induction time of 6 h. These results showed that the expression level of PmST1 on the OM of wildtype *E. coli* (BL21) and ClearColi was nearly identical with enough induction time of longer than 6 h.

The absolute amount of surface-displayed PmST1 was analyzed using a known concentration of BSA as an internal standard for the densitometry of protein bands on

SDS-PAGE. As shown in Fig. 2 (c) and Fig. 2 (d), the isolated OM of wildtype *E. coli* (BL21) and ClearColi was analyzed using the BCA total protein assay kit, to a total protein concentration of 2696.6 and 827.5 μg/mL, respectively, from the same amount of *E. coli* cells (2.4 x $10^{10}$). Likewise, the absolute amount of surface-displayed PmST1 was analyzed to have a total protein concentration of 401.2 and 168.4 μg/mL, respectively, from the same amount of *E. coli* cells (2.4 x $10^{10}$), using the densitometry of protein bands of PmST1 on SDS-PAGE gel. Additionally, the standard curve for the densitometry of BSA was made from the repeated SDS-PAGE experiments (n=6) as shown in Supplement 1. From the standard curve, the amount of BSA was observed to have a linear correlation to the densitometry results as shown in Supplement 2. By considering the isolation yield of OM fraction, the amount of surface displayed PmST1 was estimated to be 5.19 X $10^4$ molecules/cell for BL21 and 1.8 X $10^4$ molecules/cell for ClearColi. These results showed that wildtype *E. coli* (BL21) had three-fold and two-fold higher concentration for the total OM protein and PmST1, respectively, in comparison with ClearColi. However, the proportion of PmST1 among the total OM protein was estimated to be 14.9 % and 20.3 % for wildtype E. coli (BL21) and ClearColi, respectively.

**3.2 Activity assay of surface-displayed PmST1**

The enzyme activity of surface-displayed PmST1 on the OM was compared between wildtype *E. coli* (BL21) and ClearColi. For the activity assay, N-acetyl-D-lactosamine (LacNAc) was used as an acceptor and sialyl-CMP was used as a donor. In this assay configuration, CMP was released by the transfer of a sialyl group from the donor, and the acceptor was converted into the product sialyl-LacNAc (Fig. 3(a)). The enzyme reaction

was confirmed by the detection of sialyl-LacNAc in the MALDI-TOF mass spectrum. As shown in Fig. 3(b), the mass peak of the reactant (LacNAc) was observed as a sodium adduct of $[LacNAc+Na]^+$ at the m/z value of 406.85; the product was also observed as a sodium adduct of $[sialyl\text{-}LacNAc+Na]^+$ at the m/z value of 697.6. These results showed that the surface-displayed PmST1 had an enzyme activity to transfer a sialyl group to the acceptor (LacNAc).

The estimation of enzyme activity of PmST1 was performed by measurement of the concentration of CMP, which was a side product after the transfer of the sialyl group to the acceptor. As the first step, the donor substrate (CMP-Neu5Ac) and the product (CMP) were separately analyzed by using serially diluted standard samples as shown in Supplement 3, and the signal was calculated from the area of peaks. As shown in Supplement 4, the correlation between the concentration of two analytes and the calculated area showed a high linearity ($r^2$ = 0.997). For the PmST1 reaction, the peak area of CMP was observed to be quantitatively increased according to the concentration of PmST1 in samples, which represented the optical density of CMP at the wavelength of 254 nm ($OD_{254\ nm}$) as shown in Fig. 3(c). These results showed that the surface-displayed PmST1 had an enzyme activity to transfer a sialyl group to the acceptor, and the enzyme activity could be quantitatively analyzed by the measurement of a side product (CMP).

The kinetic properties of the surface-displayed PmST1 from the wildtype *E. coli* (BL21) and ClearColi were analyzed by measuring reaction rates at different conditions. As shown in Fig. 4(a), the amount of CMP was increased as the reaction time was increased from 0 to 20 min, using the isolated OM from the same number of *E. coli* cells ($2.56 \times 10^9$) at 1.05 mM of CMP-Neu5Ac and 0.157 mM of LacNAc. The slope of the graph was

calculated to be 1.93 and 2.54 μM/min for PmST1 from the wildtype *E. coli* (BL21) and ClearColi, respectively. Given that the absolute amount of PmST1 was calculated to be 401.2 and 168.4 μg/mL from the same amount of *E. coli* cells (2.4 x $10^{10}$) for wildtype *E. coli* (BL21) and ClearColi, respectively, the enzyme activity of the surface-displayed PmST1 from ClearColi was estimated to be approximately three-fold higher than that of wildtype *E. coli* (BL21). Additionally, the enzyme activity was not saturated until the reaction time of 20 min for both types of PmST1.

Thus, we fixed the reaction time for 10 min for measuring the initial reaction rates ($V_0$) with increasing amount of *E. coli* (i.e. the amount of enzyme) in an endpoint assay using HPLC. The reaction rate was also observed to be linearly increased with the amount of bacteria as shown in Fig. 4(b). The slope of graph was calculated to be 0.44 and 1.13 pM/min/*E. coli* for PmST1 from the wildtype *E. coli* (BL21) and ClearColi.

A Michaelis–Menten plot was obtained for PmST1 from the wildtype *E. coli* (BL21) and ClearColi, to compare the kinetic parameters. When the reaction time was fixed at 10 min and the amount of substrate (acceptor, LacNAc) was increased, the reaction rate ($V_0$) was observed to approach a maximum value ($V_{max}$) (Fig. 4(c)). Given that the enzyme activity of the surface-displayed PmST1 from ClearColi was higher than that of wildtype *E. coli* (BL21), the maximum rate ($V_{max}$) was also observed to be higher than that of wildtype *E. coli* (BL21). However, the $K_M$ value, which represented the concentration of substrate to reach half the maximum rate ($V_{max}$), was observed to be similar for both enzymes. An additional Michaelis–Menten plot was obtained when the amount of total proteins in the isolated OM was fixed to be same for wildtype *E. coli* (BL21) and ClearColi. Given that the absolute amount of PmST1 (in total protein) was calculated to be 401.2 (2696.6 μ

g/mL) and 168.4 μg/mL (827.5 μg/mL) from the same amount of *E. coli* cells (2.4 x $10^{10}$) for wildtype *E. coli* (BL21) and ClearColi, respectively, the absolute amount of PmST1 of ClearColi was estimated to be 1.3-fold higher than that of wildtype *E. coli* (BL21). Additionally, the enzyme activity of the surface-displayed PmST1 from ClearColi was confirmed to be 3.4-fold higher than that of wildtype *E. coli* (BL21). As shown in Fig. 4(d), the reaction rate of ClearColi in this plot was expected to be five-fold higher than that of wildtype *E. coli* (BL21).

As summarized in Table 1, the surface-displayed PmST1 from ClearColi had 3.4-fold higher $k_{cat}$ and $k_{cat}/K_M$ values in comparison with those of wildtype *E. coli* (BL21). These results indicated that PmST1 from ClearColi could have an increased reaction rate, and the higher reaction rate could be achieved at the lower substrate concentration in comparison with PmST1 from wildtype *E. coli* (BL21). Such a difference in enzyme activity was considered to be the interference of LPS on the mass transport of the donor and acceptor to PmST1 for the sialyl group transfer [38].

**Conclusions**

PmST1 is an enzyme that transfers a sialyl group of a donor substrate to an acceptor substrate called N-acetyl-D-lactosamine (LacNAc). In this study, the enzyme activity and expression level of PmST1 were compared between wildtype *E. coli* (BL21) with LPS and ClearColi with no LPS, when PmST1 was expressed on the OM. As the first step, the expression level of PmST1 on the OM of wildtype *E. coli* (BL21) and ClearColi was compared according to the IPTG induction time and was found to be nearly the same, with enough induction time of longer than 6 h. Then, the absolute amount of surface-

displayed PmST1 was analyzed using densitometry of SDS-PAGE. The isolated OM of wildtype *E. coli* (BL21) and ClearColi was analyzed using the BCA total protein assay kit, to have a total protein concentration of 2696.63 and 827.51 μg/mL, and the surface-displayed PmST1 was analyzed to have a concentration of 401.23 and 168.35 μg/mL from the same amount of *E. coli* cells (2.4 x $10^{10}$). By considering the isolation yield of OM fraction, the amount of surface-displayed PmST1 was estimated to be 5.19 X $10^4$ molecules/cell for BL21 and 1.8 X $10^4$ molecules/cell for ClearColi. The enzyme activity of PmST1 was estimated by measuring the concentration of CMP, which was a by-product after the transfer of the sialyl group to the acceptor. The enzyme activity of the surface-displayed PmST1 from ClearColi was estimated to be 3.4-fold higher than that of wildtype *E. coli* (BL21). From the Michaelis–Menten plot, the maximum rate ($V_{max}$) of the surface-displayed PmST1 from ClearColi was observed to be 3.4 fold higher than that of wildtype *E. coli* (BL21), which was reflected in the $k_{cat}$ values of wildtype *E. coli* (0.186 $s^{-1}$) and PmST1 in ClearColi (0.640 $s^{-1}$). This indicates that the $K_M$ values should be similar, which is consistent with the observation that $K_M$ values for wildtype *E. coli* (BL21) and ClearColi were 0.55 and 0.56 mM, respectively. These results implied that such a difference in enzyme activity could be attributed to the interference of LPS on the mass transport of the donor and the acceptor to PmST1 for the sialyl group transfer.

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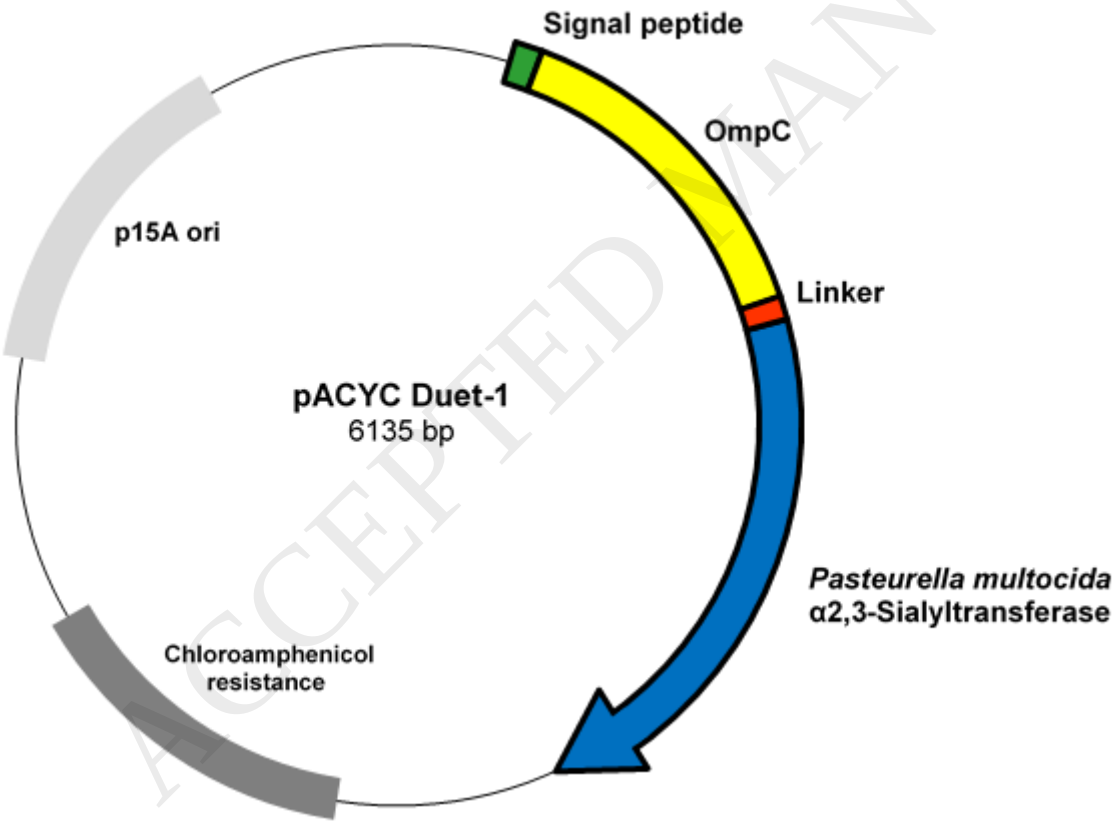
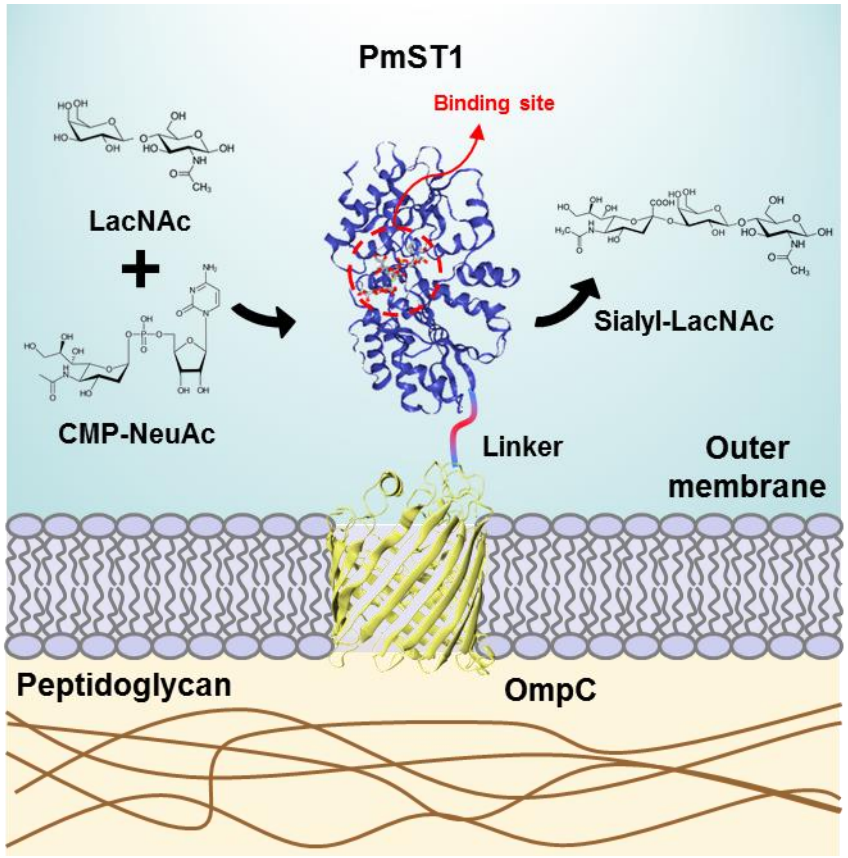
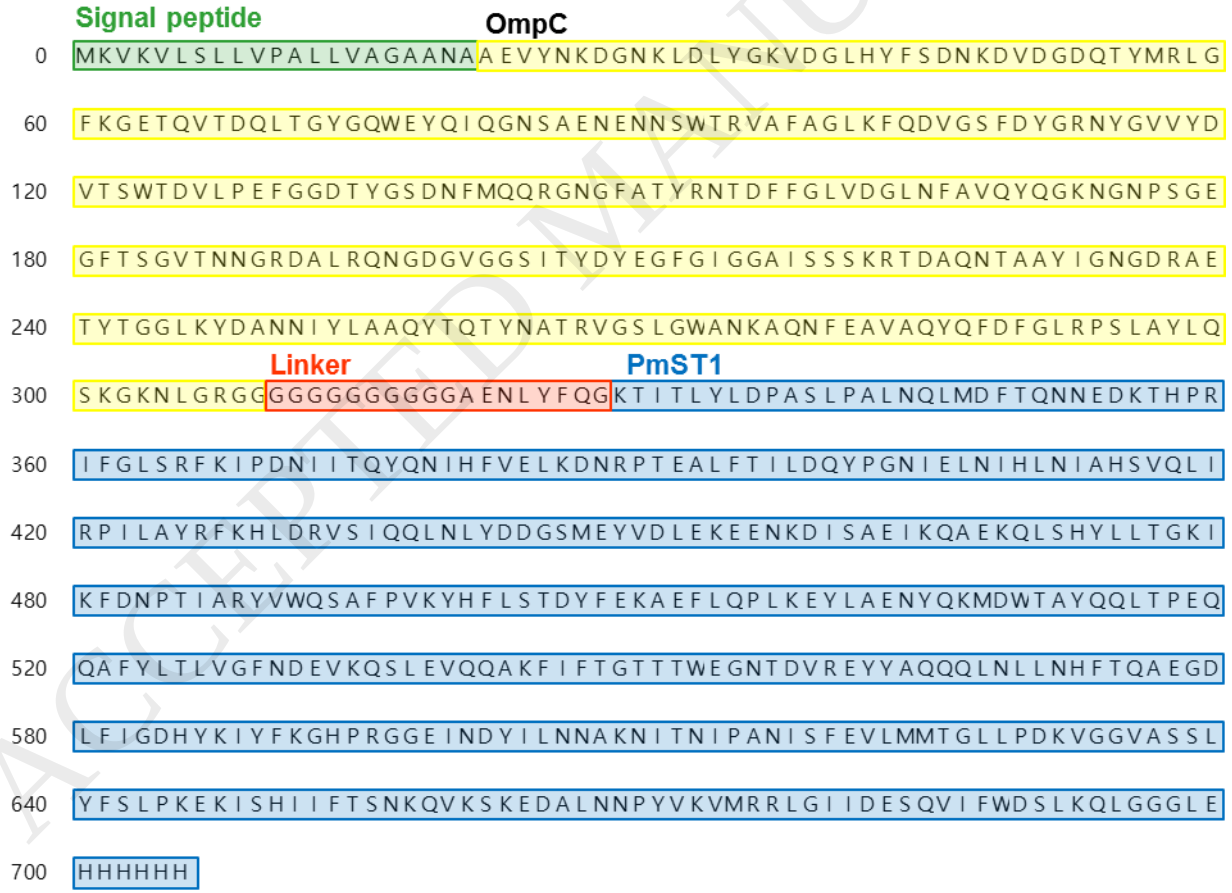
**Fig 1. (a) Surface display scheme**

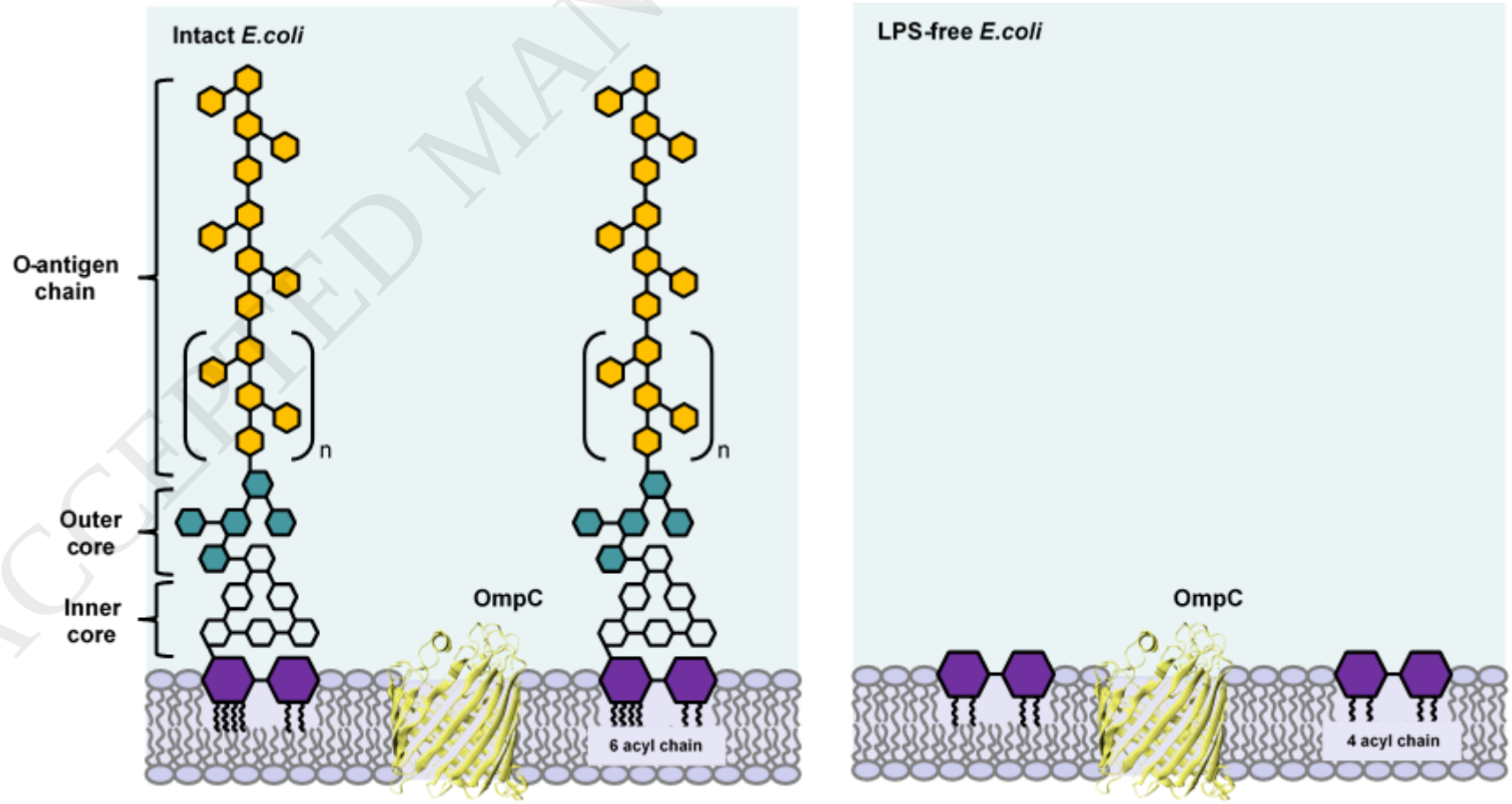


## (b) OmpC-PmST1 Sequence



**(c) LPS-free E.coli outer membrane scheme**



**(d) SDS-PAGE of Fusion protein**

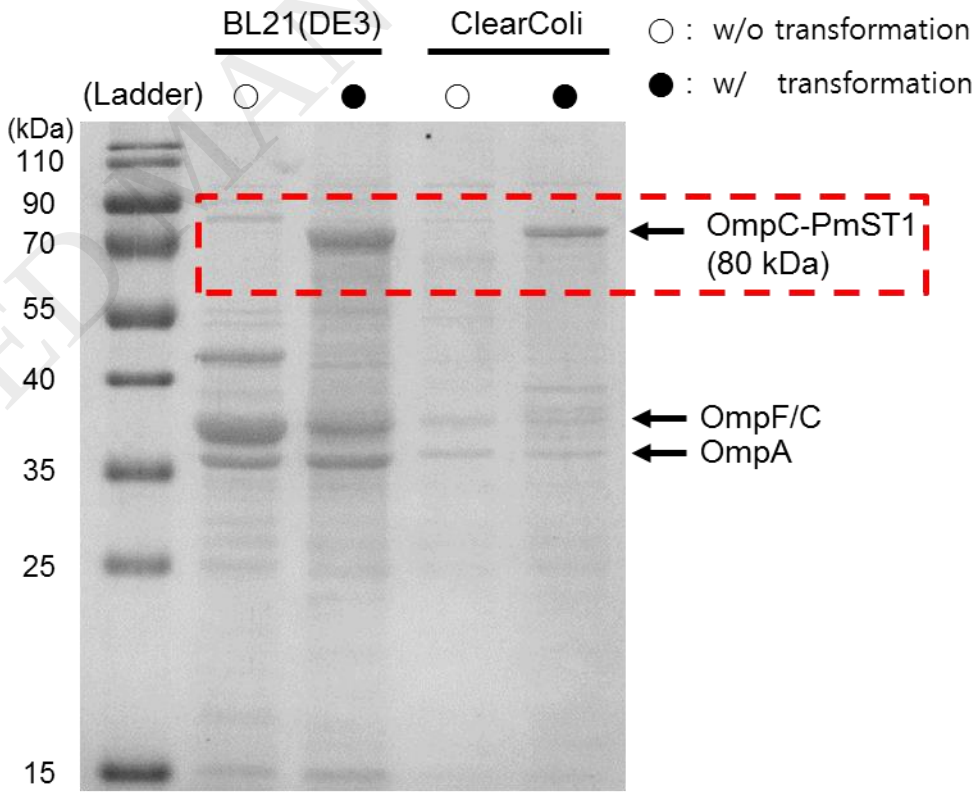
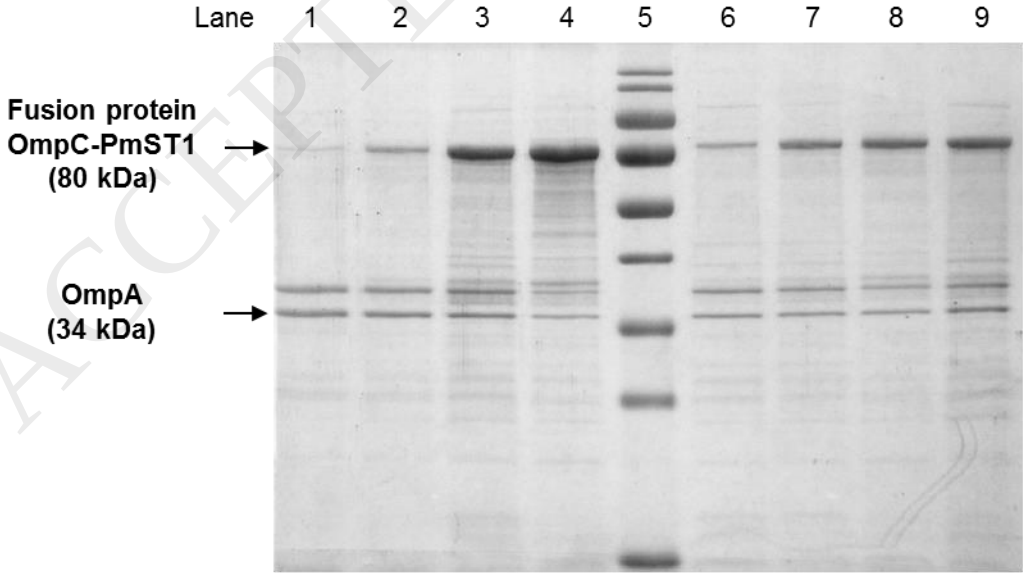
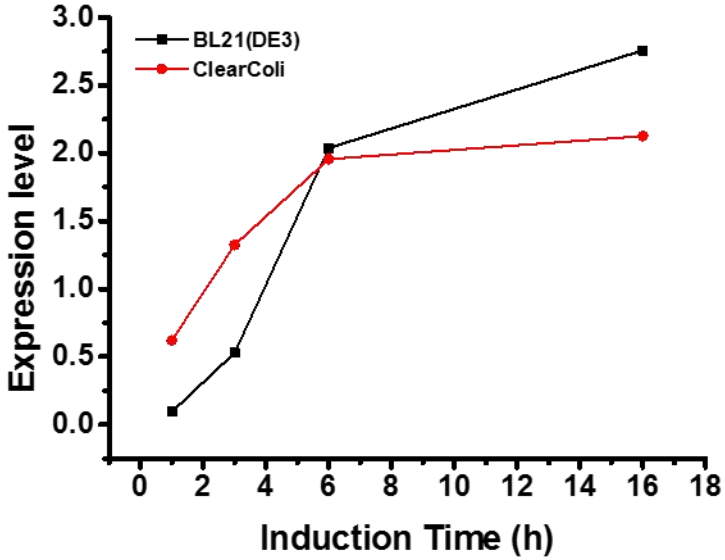



Fig. 1. Surface display of sialyltransferase from *Pasteurella multocida* (PmST1) as a fusion protein of OmpC. (a) Schematic view of the surface display vector of PmST1 (pACYC Duet-1) and the surface-displayed PmST1. Yellow domain from *Escherichia coli* OmpC (PDB

ID: 2J1N) and blue domain from *Pasteurella multocida* sialyltransferase (PDB ID: 2EX1) (b) Amino acid sequence of PmST1 inserted to the surface display vector (pACYC Duet-1). (c) Comparison of the outer membrane (OM) structure of wildtype *Escherichia coli* (BL21) with lipopolysaccharide (LPS) and ClearColi without LPS. (d) SDS-PAGE of surface-displayed PmST1 on the OM structure of wildtype *E. coli* (BL21, lane 2) and ClearColi (lane 4).

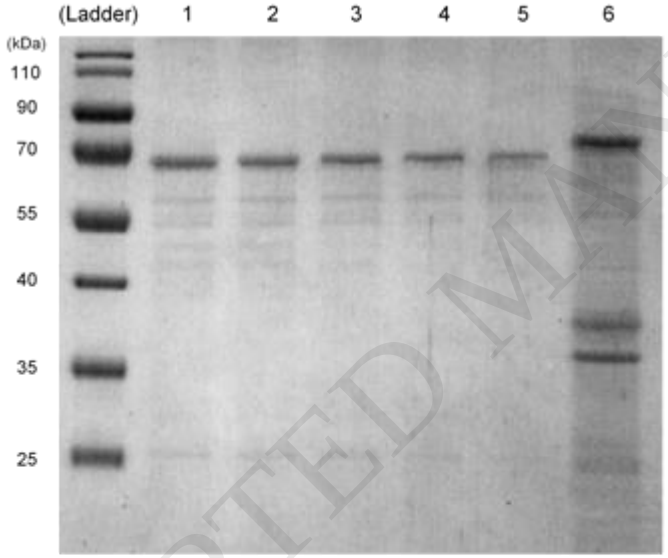
**Fig 2. (a)** **(b)**

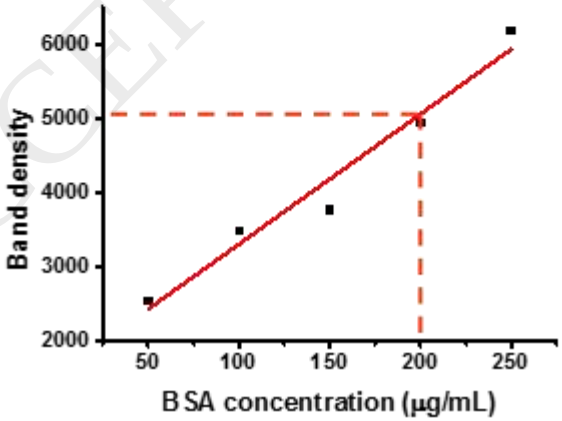


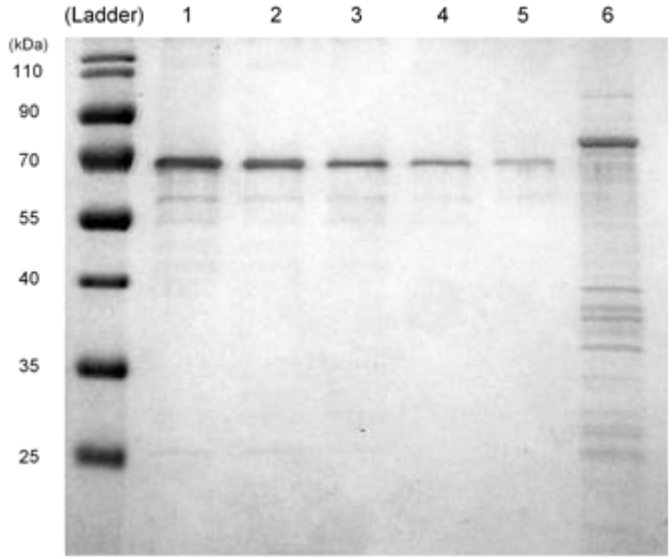



**(c)**

**(d)**



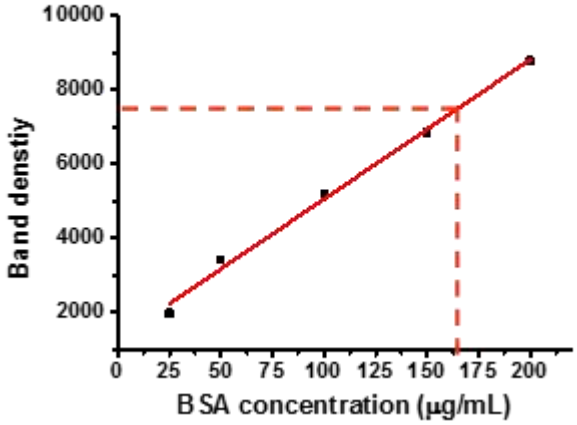
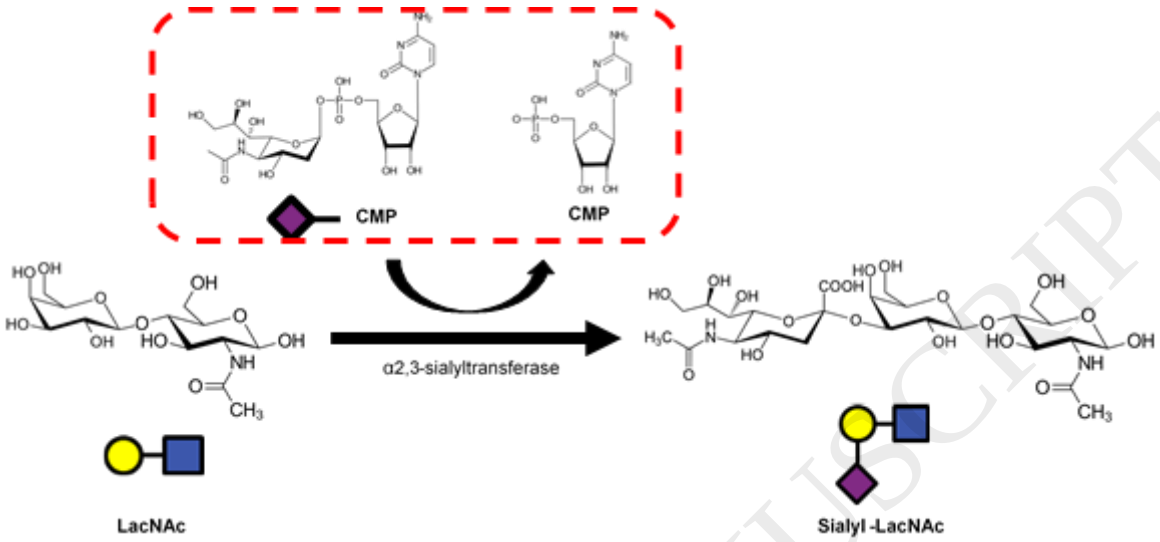



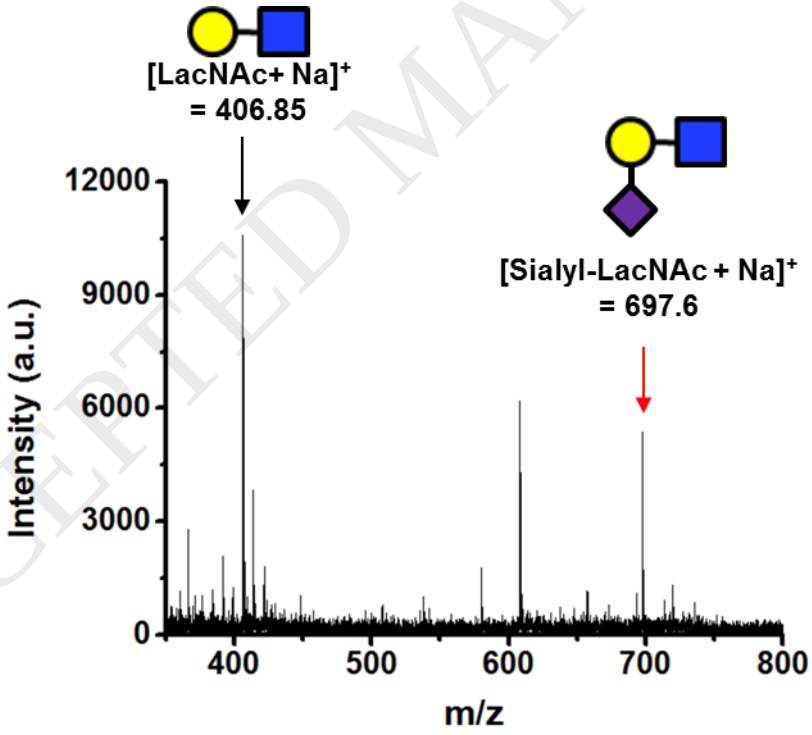
Fig. 2. Comparison of expression level of PmST1 on the OM structure of wildtype *E. coli* (BL21) and ClearColi. (a) SDS-PAGE of surface-displayed PmST1 according to incubation times of 1, 3, 6, and 16 h for wildtype *E. coli* (BL21, lanes 1–4) and ClearColi (lanes 6–9). (b) Expression profile of wildtype *E. coli* (BL21) and ClearColi. (c) Densitometry of surface-displayed PmST1 on the OM of wildtype *E. coli* (BL21). (d) Densitometry of surface-displayed PmST1 on the OM of ClearColi.

**Fig 3.**

**(a)**




**(b)**



**(c)**

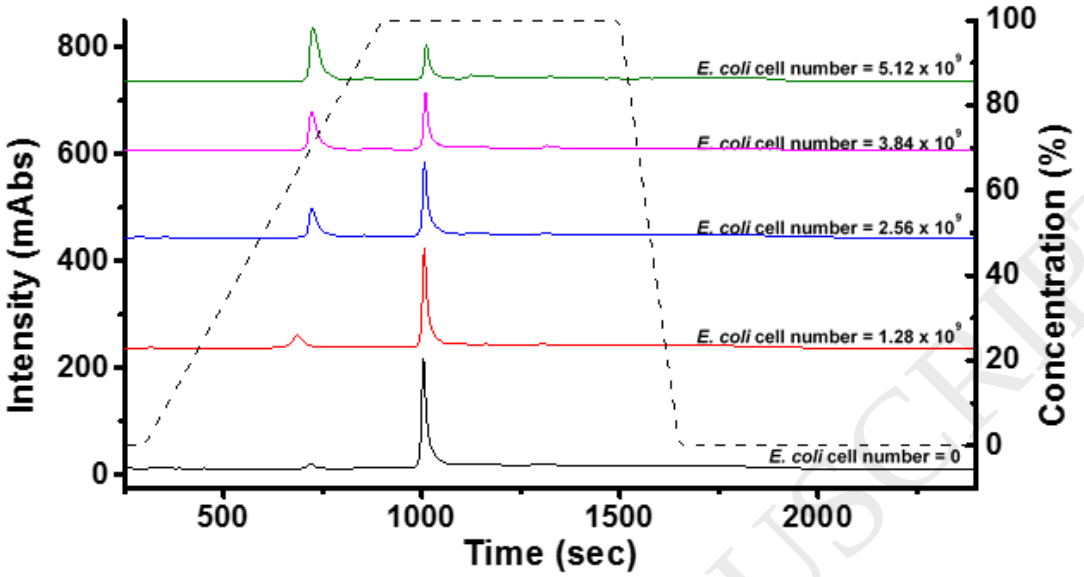
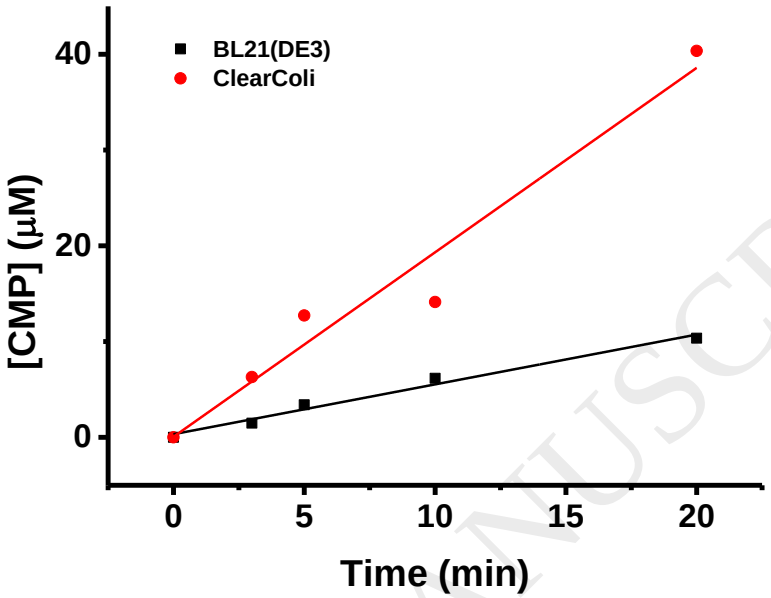



Fig. 3. Enzyme activity assay of PmST1. (a) Assay configuration of PmST1, using LacNAc as an acceptor and sialyl-CMP as a donor. (b) MALDI-TOF mass spectrum of the reaction mixture. (c) Optical density profiles of CMP for the quantitative analysis of the enzyme activity of PmST1.

Fig 4.

(a)

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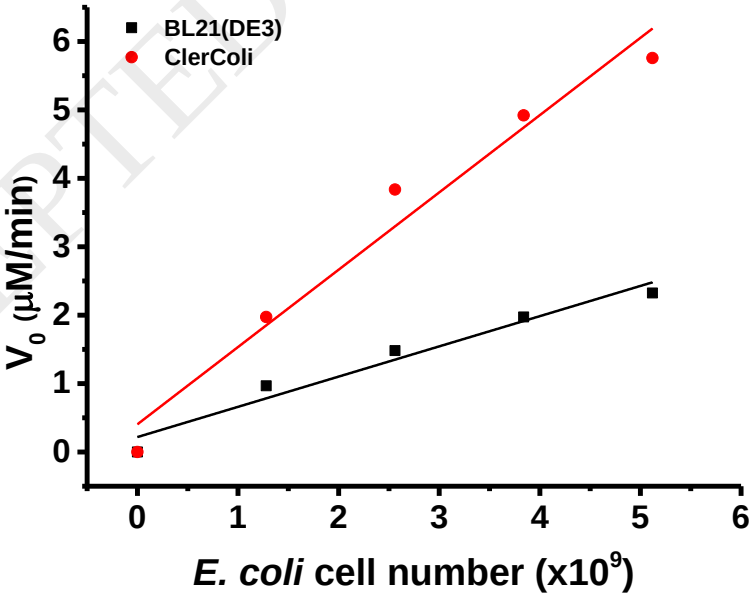
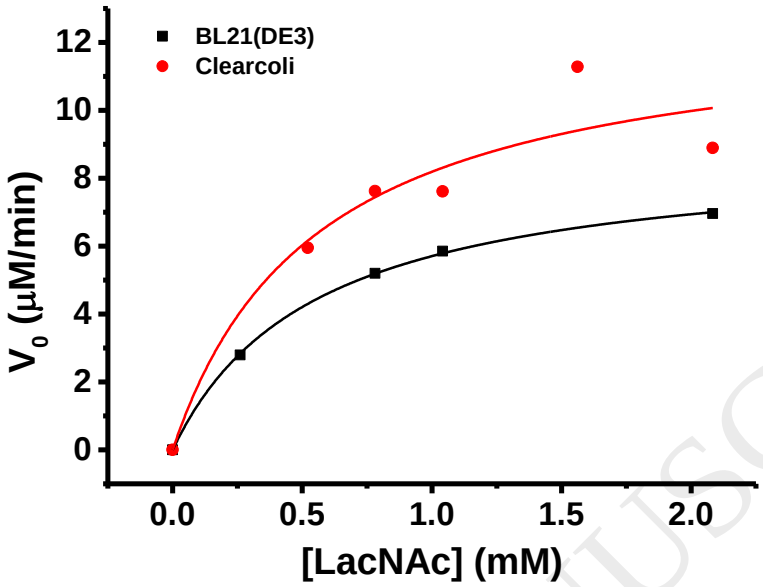


(c)




(d)

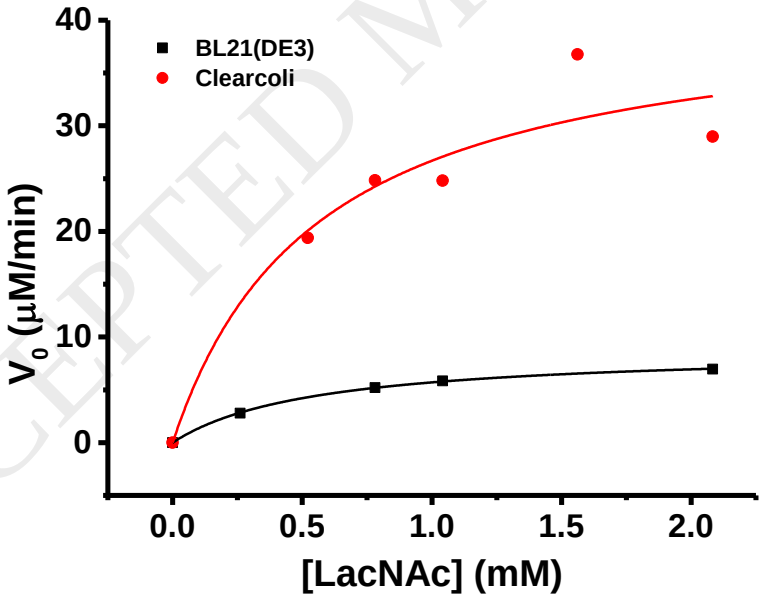



Fig. 4. Kinetic assay of the enzyme activity of PmST1. (a) Product formation profile according to reaction time. (b) Reaction rate profile according to the number of *E.*

*coli* cells with surface-displayed PmST1 at the reaction time of 10 min. (c) Michaelis–Menten plot with the same number of wildtype *E. coli* (BL21) and ClearColi. (d) Michaelis–Menten plot with the same concentration of the total OM protein for wildtype *E. coli* (BL21) and ClearColi.

## Table legends

Table 1. Kinetic parameter of surface displayed sialyltransferase from *Pasteurella multocida* (PmST1).

Table 1. Kinetic parameters of surface displayed PmST1.

| | $V_{max\_A}$* (μM·min) | $V_{max\_B}$** (μM·min) | $k_{cat}$ ($s^{-1}$) | $K_m$ (mM) | $k_{cat}/K_m$ ($s^{-1}mM^{-1}$) |
|---|---|---|---|---|---|
| OmpC-PmST1 (BL21 strain) | 8.86 | 8.86 | 0.186 | 0.55 | 0.337 |
| OmpC-PmST1 (ClearColi™ strain) | 12.78 | 41.63 | 0.640 | 0.56 | 1.14 |

*** $V_{max\_A}$ :** Maximum velocity for the same number of *E. coli* cells

**** $V_{max\_B}$ :** Maximum velocity for the same concentration of outer membrane proteins